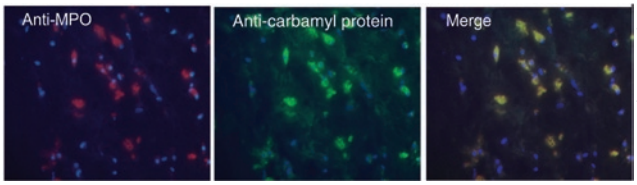
Kidney International (2008) **73**, 9–10. doi:10.1038/sj.ki.5002709

Protein carbamylation links uremia, inflammation, and atherogenesis



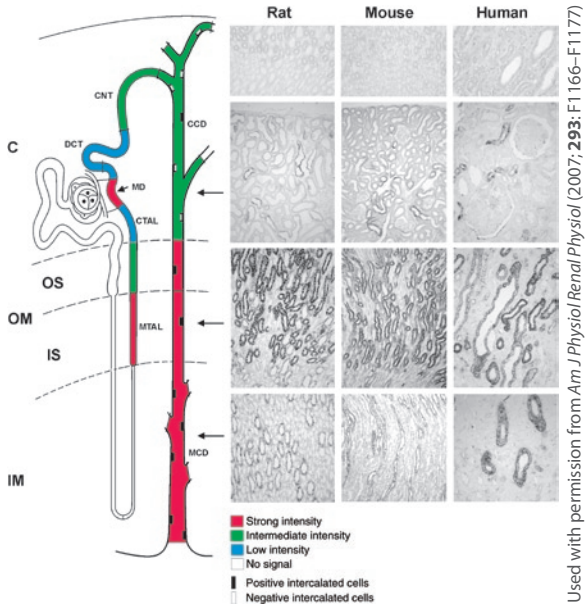
Human carotid atherosclerotic plaque immunostained with antibodies against MPO (left) or carbamyl proteins (middle). The merged image (right) reveals colocalization of MPO and carbamyl proteins. Nuclei were stained with DAPI.

It has long been known that the urea-generated molecule cyanate can modify lysine residues and generate homocitrulline (also known as ε-carbamyllysine) in a process called carbamylation. This carbamylation of amino acids, proteins, and other molecules changes their structure and function, thus modifying the activity of enzymes, cofactors, hormones, low-density lipoproteins, antibodies, receptors, and transport proteins. Antibodies specific for homocitrulline locate carbamylated proteins in neutrophils, monocytes, and erythrocytes. Cyanate spontaneously transformed from urea increases as renal function decreases; carbamylated proteins have been found in renal tissue from uremic patients but not in normal transplanted kidneys. Carbamylated proteins isolated from uremic subjects have been shown to have decreased biological activity, and it has been hypothesized that they posses pro-atherosclerotic activities, perhaps contributing to the severe atherosclerosis of patients with renal failure. Because of the extremely low levels of protein carbamylation when normal plasma levels of urea are present, the potential involvement of this pathway in atherosclerotic vascular disease in the absence of renal failure has not until now been explored. In a recent article, Wang *et al.* report a biochemical pathway involving the enzyme myeloperoxidase (MPO) that causes protein carbamylation. MPO is a heme protein abundant in neutrophils, monocytes, and certain tissue macrophages, such as those found in human atheroma. The authors found that MPO promotes protein carbamylation during inflammation and within human atherosclerotic plaque (Figure). Further, like low-density lipoprotein isolated from uremic patients, MPO-carbamylated lipoproteins had atherogenic properties. Wang *et al.* also found that blood levels of protein-bound homocitrulline correlated with increased cardiovascular risk in patients. This study newly identifies a biochemical pathway that is likely to be important in the promotion of atherogenesis and that may clarify the mechanisms responsible for the severity of this disease in patients with end-stage renal failure. (*Nat Med* 2007; **13**: 1176–1184)

Juan Oliver

Vasopressin V_2 receptor expression along the nephron

In the kidney, two receptors for arginine vasopressin (AVP), V_{1a} and V_2 receptors ($V_{1a}R$ and V_2R), have been characterized. $V_{1a}R$ is localized in the renal vasculature and glomeruli and mediates the vasopressor effect of AVP. The tubular antidiuretic effect of AVP is mediated by the V_2R and adenylyl cyclase-dependent cAMP signaling. The collecting duct is the principal target for the antidiuretic action of AVP, but data indicate that AVP has functions in the distal tubule and the thick ascending limb (TAL). Despite the prominent functional role of V_2R-mediated effects of AVP in the kidney, the exact location of this receptor in different nephron segments and cell types remains somewhat unclear. In a new study, Mutig *et al.* present a detailed description of the distribution of V_2R mRNA and protein in the different nephron segments in mice, rat and human kidneys. The different cell types of the renal tubules were identified by specific markers. They found solid expression of V_2R mRNA in the medullary TAL (MTAL), macula densa, connecting tubule, and cortical and medullary collecting duct, and weaker expression in the cortical TAL and distal convoluted tubule, in all three species (Figure). Previous studies have shown that in the TAL, AVP stimulates transepithelial NaCl transport by increasing abundance and phosphorylation of Na^+-K^+-$2Cl^-$ cotransporter type 2 (NKCC2). The authors found that macula densa cells not only had high levels of V_2R mRNA but also constitutively showed strong NKCC2 phosphorylation.

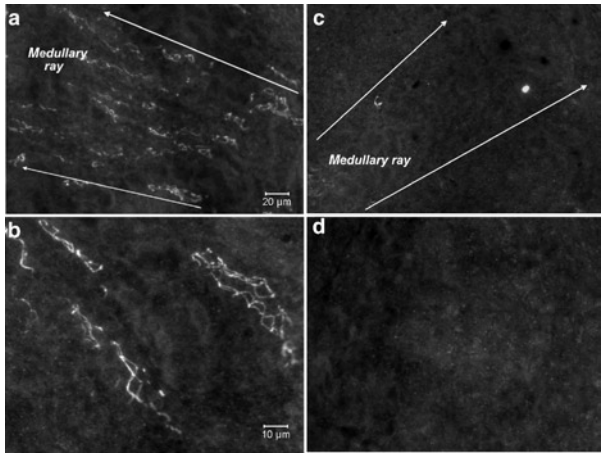


Zonal and segmental distribution of baseline vasopressin V_2 receptor (V_2R) mRNA expression in rat, mouse, and human renal epithelia. Kidney zones are cortex (C), outer medulla (OM) with outer stripe (OS) and inner stripe (IS), and inner medulla (IM). Tubule segments are medullary and cortical thick ascending limb (MTAL and CTAL), macula densa (MD), distal convoluted tubule (DCT), connecting tubule (CNT), and cortical and medullary collecting duct (CCD and MCD).

These results suggest that in all three species comparably significant effects of vasopressin-induced V_2R signaling in MTAL and in connecting tubule/collecting duct principal cells occur. Strong V_2R expression in the macula densa is an intriguing finding that may improve understanding of tubuloglomerular feedback. (*Am J Physiol Renal Physiol* 2007; **293**: F1166–F1177)
Juan Oliver

Claudin-16 and magnesium renal handling

The human renal disorder familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is characterized by renal Mg^{2+} and Ca^{2+} wasting, progressive impairment of renal function, nephrocalcinosis, and chronic renal failure. FHHNC is genetically linked to mutations in the gene of claudin-16 (CLDN16), which is expressed exclusively in the kidney. The claudins comprise a large gene family encoding structural components of the tight junction, the principal regulators of paracellular permeability that, needless to say, have tight junctions that play important roles in regulating ion reabsorption in the kidney. But the molecular mechanisms underlying the renal dysfunction in FHHNC remain unknown. Using lentiviral transgenic RNA interference, Hou *et al.* now report generation of CLDN16-deficient transgenic mouse lines and describe the physiological function of CLDN16. In the TAL of the nephron, electrogenic NaCl reabsorption generates a lumen-positive transepithelial potential, which drives the reabsorption of Mg^{2+} and Ca^{2+}. It is a diffusion potential between luminal and basolateral extracellular spaces if they are separated by cation-selective tight junctions. The authors show that the loss of CLDN16 (Figure) caused tight junctions in the TAL to lose the cation selectivity, leading to dissipation of the lumen-positive potential with concomitant loss of the driving force for Mg^{2+} reabsorption. Claudin-16 knock-down mice exhibited chronic renal wasting of magnesium and calcium and developed renal nephrocalcinosis. Thus, claudin-16 plays a key role in maintaining the paracellular cation selectivity of the TALs of the nephron by forming a nonselective paracellular cation channel, rather than a previously proposed selective Mg^{2+}/Ca^{2+} channel. This model clarifies the pathogenesis of FHHNC and suggests that analysis of tight junction proteins may illuminate renal electrolyte handling and disorders. (*J Biol Chem* 2007; **282**: 17114–17122)
Qais Al-Awqati



Hou *et al.*/*J Biol Chem*

CLDN16 in the kidney. Sections from wild-type mouse kidneys (**a** and **b**) show CLDN16 localization in the tight junctions of tubules in medullary rays (that is, the TAL). In CLDN16 knock-down mouse kidneys (**c** and **d**), CLDN16 staining disappears.

Hypertension in mice with deletion of calcitonin gene-related peptide receptor

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide produced in the neural body of dorsal root ganglion cells and released from sensory nerve endings. There are two isoforms of CGRP, α and β. Whereas αCGRP is produced mainly in the nervous system, βCGRP is produced in neuronal tissues but also in enteric nerves and in immune cells. Pharmacologically, αCGRP possesses very potent vasodilatory activity. Most blood vessels are surrounded by a dense perivascular neural network, suggesting an important role for CGRP in vascular regulation. It has long been suspected that αCGRP and βCGRP are involved in arterial pressure control, but their precise functional role and development remain unclear. In addition, development of several αCGRP-deficient mice has provided conflicting results. The CGRP receptor consists of a seven-transmembrane G protein-coupled receptor called the calcitonin receptor-like receptor (CLR) and a single membrane-spanning protein called receptor activity-modifying protein-1 (RAMP1), which determines the specificity of the CGRP receptor. To clarify the physiological functions of CGRP mediated through the CLR/RAMP1 receptors *in vivo*, Tsujikawa *et al.* generated RAMP1-deficient mice. They found that $RAMP1^{-/-}$ mice exhibited severe hypertension, which resulted mainly from a vasodilatory disorder caused by deficient αCGRP signal transduction. In addition, $RAMP1^{-/-}$ mice exhibited a significant increase in serum CGRP and proinflammatory cytokine levels after lipopolysaccharide administration. These results indicate that CGRPs transduce physiologically critical signals in the regulation of blood pressure, and that CLR/RAMP1 receptors are involved in the inflammatory response. (*Proc Natl Acad Sci USA* 2007; **104**: 16702–16707)
Juan Oliver